

CLAIMS

1. A transgenic insect or transgenic insect cell, wherein the level of expression of *dhr96* or a homologue thereof has been reduced relative to the level of expression of *dhr96* or said homologue in a non-transgenic insect or non-transgenic insect cell.
2. A transgenic insect or transgenic insect cell according to claim 1 wherein the homologue is from an insect selected from the group consisting of *Drosophila* sp., *Bombyx mori*, *Tribolium castaneum*, *Aedes aegyptii*, *Anopheles gambiae*, *Anopheles albimanus*, *Anopheles stephensi*, *Ceratitis capitata*, *Pectinophora gossypiella*, *Helicoverpa zea*, *Bactrocera dorsalis*, *Anastrepha suspense*, *Musca domestica*, *Stomoxys calcitrans*, and *Lucilia cuprina*.
3. A transgenic insect or transgenic insect cell according to claim 1 or claim 2 wherein the transgenic insect or transgenic insect cell is selected from the group of insects consisting of: *Drosophila* sp., *Bombyx mori*, *Tribolium castaneum*, *Aedes aegyptii*, *Anopheles gambiae*, *Anopheles albimanus*, *Anopheles stephensi*, *Ceratitis capitata*, *Pectinophora gossypiella*, *Helicoverpa zea*, *Bactrocera dorsalis*, *Anastrepha suspense*, *Musca domestica*, *Stomoxys calcitrans*, and *Lucilia cuprina*.
4. A transgenic insect or transgenic insect cell according to claim 3 which is or comes from a species of *Drosophila*.
5. A transgenic insect or transgenic insect cell according to claim 4 which is or comes from *D. melanogaster*.
6. A transgenic insect or transgenic insect cell according to any one of the preceding claims wherein the level of expression of *dhr96* or said homologue has been reduced through RNAi.

7. A transgenic insect or transgenic insect cell according to any one of claims 1 to 5 wherein the level of expression of *dhr96* or said homologue has been reduced through insertional mutagenesis.
- 5 8. A transgenic insect or transgenic insect cell according to any one of claims 1-5 wherein the level of expression of *dhr96* or said homologue has been reduced through homologous recombination.
9. A DNA construct comprising a first DNA sequence encoding a fragment of *dhr96*
10 or homologue thereof and a second DNA sequence which is the reverse complement of the first DNA sequence, wherein the first and second DNA sequences are present on the same strand of DNA and are operably linked to a promoter region and optionally a terminator region
- 15 10. A DNA construct according to claim 9 wherein the first and second DNA sequences are separated by a third DNA sequence which acts a spacer sequence between the first and second DNA sequences.
11. A DNA construct according to claim 9 or 10 wherein the first DNA encodes a
20 fragment of *dhr96* from *D. melanogaster*.
12. A DNA construct according to claim 11 which comprises bases 58-661 of SEQ ID NO 1, or the reverse complement thereof.
- 25 13. A method of producing a transgenic insect as defined in any one of claims 1-6, which comprises:
 - (i) transforming a first insect with a first DNA construct according to any one of claims 9 to 12 wherein the promoter region comprises an inducible promoter;
 - 30 (ii) transforming a second insect with a second DNA construct encoding a protein that is capable of inducing expression from the inducible promoter in the first DNA construct;
 - (iii) crossing the transformed insects resulting from steps (i) and (ii) to obtain progeny;

5 wherein a population of the progeny resulting from step (iii) comprises both the first and the second DNA constructs and when the protein encoded in the second DNA construct is expressed, the level of expression of *dhr96* or said homologue is reduced in said population of the progeny relative to the level of expression of *dhr96* or said homologue in a non-transgenic insect.

14. A method of producing a transgenic insect as defined in any one of claims 1-6, which comprises:
- 10 (i) transforming a first insect with a first DNA construct according to any one of claims 9 to 12 wherein the promoter region comprises an inducible promoter; and,
- (ii) crossing the transformed insect resulting from step (i) with an insect that expresses an inducing protein that is capable of inducing expression from the inducible promoter in the first DNA construct, to obtain progeny;
- 15 wherein a population of the progeny resulting from step (ii) comprise the first DNA construct and express the inducing protein so that the level of expression of *dhr96* or said homologue is reduced in said population of the progeny relative to the level of expression of *dhr96* or said homologue in a non-transgenic insect.
- 20 15. A method of identifying or verifying the ability of a compound to act as an insecticide, which comprises:
- (i) placing a transgenic insect or transgenic insect cell according to any one claims 1 to 8 in contact with the compound;
- 25 (ii) assessing the transgenic insect or transgenic insect cell that has been placed in contact with the compound in step (i) for any deleterious effect on the transgenic insect or transgenic insect cell;
- wherein the presence of a deleterious effect is indicative that said compound is capable of acting as an insecticide.

- 30 16. A method of identifying the ability of a compound to act as a pro-insecticide, which comprises:
- (i) placing a transgenic insect or transgenic insect cell according to any one claims 1 to 8 in contact with the compound;

(ii) assessing said transgenic insect or transgenic insect cell that has been placed in contact with the compound in step (i) for any deleterious effect on the transgenic insect or transgenic insect cell;

(iii) comparing the effects observed in step (ii) to the effects observed on a) a non-transgenic insect or non-transgenic insect cell and/or b) a transgenic insect or transgenic insect cell wherein the level of expression of *dhr96* or homologue thereof has been increased in comparison to the level of expression in a non-transgenic insect or non-transgenic insect cell;

wherein the presence of a deleterious effect on a) a non-transgenic insect or non-transgenic insect cell and/or b) a transgenic insect or transgenic insect cell wherein the level of expression of *dhr96* or homologue thereof has been increased in comparison to the level of expression in a non-transgenic insect or non-transgenic insect cell coupled to the absence of a deleterious effect on transgenic insect or transgenic insect cell in step (ii) is indicative that said compound is capable of acting as a pro-insecticide.

17. A method of identifying a compound that regulates expression of *dhr96* or a homologue thereof, which comprises:

(i) culturing in a suitable growth medium a population of cells transformed with a DNA construct comprising a reporter gene operably linked to the promoter region of *dhr96* or a homologue thereof

(ii) incubating said compound with a population of cells according to step (i);

(iii) determining the level of expression of said reporter gene in cells that have been incubated with said compound; and

(iv) determining the level of expression of said reporter gene in a population of cells according to step (i);

wherein, a difference in the levels of reporter gene expression determined at (iii) and (iv) is indicative that said compound is capable of acting as a regulator of expression of said insect nuclear hormone receptor gene.

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18. A method of identifying a compound that regulates expression of *dhr96* or homologue thereof, which comprises:

- (i) culturing on a suitable growth medium an insect transformed with a DNA construct comprising a reporter gene operably linked to the promoter region of *dhr96* or a homologue thereof
- (ii) incubating said compound with transformed insect as described in step (i);
- (iii) determining the level of expression of said reporter gene in the transformed insect of step (ii); and
- (iv) determining the level of expression of said reporter gene in a transformed insect as described in step (i);
- wherein, a difference in the levels of reporter gene expression determined at (iii) and (iv) is indicative that said compound is capable of acting as a regulator of expression of said insect nuclear hormone receptor gene.
19. A DNA construct comprising a first DNA sequence encoding the ligand binding domain of DHR96 and a second DNA sequence encoding a heterologous eukaryotic DNA binding domain, operably linked to a promoter and optionally a terminator region.
20. A DNA construct according to claim 19, wherein the first DNA sequence additionally encodes the hinge region and the F domain of DHR96.
21. A DNA construct according to claim 20, wherein the first DNA sequence encodes a peptide having the amino acid sequence given in SEQ ID NO 15.
22. A DNA construct according to claim according to any one of claims 19 to 21 wherein the heterologous eukaryotic DNA binding domain is the GAL4 DNA binding domain.
23. A DNA construct according to any one of claims 19 to 22, wherein the promoter is the *hsp70* promoter sequence.
24. A transgenic insect or transgenic insect cell comprising a first DNA construct according to any one of claims 22 to 23, and a second DNA construct, said

second DNA construct comprising a GAL4 responsive element situated upstream of and linked to a reporter gene.

25. A transgenic insect or transgenic insect cell according to claim 24 wherein said
5 reporter gene is a fluorescent reporter gene selected from the group consisting of
amfp486, asfp595, zfp538 and zfp506.
26. A method of identifying the ability of a compound to act as a regulator of DHR96
function, which comprises:
- 10 (i) contacting a transgenic insect or transgenic insect cell of claim 24 or claim
25 with the compound; and
- (ii) measuring the level of reporter gene expression in said transgenic insect or
transgenic insect cell,
wherein the level of reporter gene expression correlates with the ability of the
15 compound to regulate DHR96 function.
27. A transgenic insect or transgenic insect cell of a first insect species comprising a
nuclear hormone receptor gene from a second insect species, wherein said nuclear
hormone receptor gene is *dhr96* or a homologue thereof.